Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 75-78, 81-84, 86-103, 105-107 and 109-145 are pending in the application, with claims 75, 102, 103 and 125 being the independent claims. Claim 79 is sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 137-145 are sought to be added. These changes are believed to introduce no new matter.

It is believed that the amendments presented above will place the application in condition for allowance and/or in better form for appeal. *See* 37 CFR § 1.116(a). The amended and new claims do not raise any new issues that would require further consideration and/or search. Thus, Applicants believe that, in accordance with 37 CFR § 1.116(a), the amended and new claims presented above should be entered after final.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Support for Amended and New Claims

Support for the amended and new claims can be found throughout the specification, for example at page 21, line 23 through page 22, line 25.

II. Claim Objections

Claim 79 was objected to under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. *See* Paper No. 22, page 3. It was noted that claim 79 specifies that the RNA-dependent RNA polymerase is of viral origin but that claim 79 depends from claim 75 which specifies that the RNA-dependent RNA polymerase is of alphaviral origin. *See id.* Applicants have cancelled claim 79, thereby obviating this objection.

Claims 120-122 were objected to for the recitation of the phrase "at least DNA molecule" in claim 120. Claim 120 has been amended to recite "at least one DNA molecule." Thus, this objection has been fully accommodated and should be withdrawn.

Claims 128, 132 and 136 were objected to for reciting "Easter" rather than "Eastern," and for not capitalizing the 'm' in "morgan." Claims 128, 132 and 136 have been amended to correct these typographical errors. Thus, this objection has been fully accommodated and should be withdrawn.

III. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

A. Written Description

Claims 75-79, 81-84, 86-101, 103, 105-107 and 109-136 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the

claimed invention. *See* Paper No. 22, pages 3-4. Applicants respectfully traverse this rejection.

To support this rejection, the Examiner stated:

> Applicant discloses a single open reading frame encoding a Sindbis virus RNA-dependent RNA polymerase. This polymerase comprises a P726S nsP2 mutation in combination with a G153E nsP4 mutation. The P726S nsP2 and G153E nsP4 mutations are the structural features which are required to render the Sindbis virus polymerase both temperature sensitive and non-cytopathic. . . Temperature sensitivity and non-cytopathicity are the necessary common attributes which the polymerase must possess in order to qualify as a member of the claimed genus. However, the specification has failed to disclose what mutations are required to render any other RNA-dependent RNA polymerase both temperature sensitive and non-cytopathic, or what other mutations could confer this phenotype on the Sindbis virus polymerase.

Paper No. 22, pages 4-5. Applicants respectfully disagree with this conclusion.

Nevertheless, solely to expedite prosecution, Applicants note that independent claims 75, 103 and 125 have been amended to specify that, for the alphavirus replicases included within these claims, non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase. According to the Federal Circuit, the written description of a genus may be achieved by recitation of, *inter alia*, structural features common to members of the genus, which features constitute a substantial portion of the genus. *See Regents of the University of California v. Eli Lilly*, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In the present specification, it is noted that non-cytopathicity and temperature sensitivity can be conferred in an alphaviral replicase by mutating, for example, the nsP2 and nsP4 genes, respectively. *See* specification at page 21, line 23 through page 22, line 25. Although mutations in nsP2 and nsP4 are exemplified,

it would be appreciated by persons of ordinary skill in the art that non-cytopathicity and temperature sensitivity may also be achieved by mutations in other non-structural alphaviral genes. In addition Applicants note that alphaviruses generally share a high degree of sequence homology with one another. *See* Applicants' Amendment and Reply filed on July 31, 2001, page 10. Thus, persons of ordinary skill in the art would conclude that Applicants were in possession of the genus of alphavirus replicases recited in the present claims.

In view of the amendments to claims 75, 103 and 125 and the discussion presented above, Applicants respectfully request that the rejection of claims 75-79, 81-84, 86-101, 103, 105-107 and 109-136 under 35 U.S.C. § 112, first paragraph, for insufficient written description, be reconsidered and withdrawn.

B. Enablement

Claims 75-79, 81-84, 86-101, 103, 105-107 and 109-136 were rejected under 35 U.S.C. § 112, first paragraph, because, according to the Examiner:

> the specification, while being enabling for a DNA molecule encoding the Sindbis virus non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase with P726S nsP2 and G153E nsP4 mutations encoded by SEQ ID NO:1, does not reasonably provide enablement for DNA molecules encoding any other alphavirus non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, or for the use *in vivo* of any alphaviral particle, RNA or DNA.

Paper No. 22, pages 7-8. Applicants respectfully traverse this rejection.

The Examiner's first basis for this rejection is that:

> [c]laims 86, 93-101, 109 and 116-124 are methods of using the nucleic acids of the invention either *in vivo* or *in vitro*.

> The specification asserts no utility for using these nucleic acids *in vivo* other than gene therapy. The specification fails to enable the general practice of gene therapy for the reasons given in Paper No. 11.

Paper No. 22, page 8. Applicants disagree with this statement. Nevertheless, solely to expedite prosecution, claims 86, 93, 97-101, 109, 116, 117 and 120-124 have been amended to specify that DNA molecules, RNA molecules or polynucleotides are introduced into a host cell *in vitro*. Thus, the enablement rejection, insofar as it relates to the inclusion of methods which involve use of the nucleic acid molecules of the invention *in vivo*, has been fully accommodated.

The Examiner has also asserted that the invention is not enabled because the specification discloses only one example of a Sindbis virus temperature-sensitive, non-cytopathic RNA dependent RNA polymerase. *See* Paper No. 22, page 10. The Examiner acknowledged that it would be simple to construct nucleic acid molecules having both mutations that confer temperature sensitivity and mutations that confer non-cytopathicity. *See* Paper No. 22, pages 8-9. Nevertheless, the Examiner stated that "the characteristics of these novel polypeptides would be highly unpredictable." Paper No. 22, page 9. The basis for this assertion is that, in the Examiner's view, "it is not currently possible to accurately predict the effects of mutations on the functions of proteins." *Id.* The Examiner also stated:

> Applicant has disclosed mutations only of a Sindbis virus polymerase, whereas the claims encompass RNA-dependent RNA polymerases from all alphaviruses. One of skill in the art could not predict which, if any, of these polymerases could be mutated to be appropriately temperature sensitive and non-cytopathic, or what mutations would be required for this.

Paper No. 22, page 10.

Applicants submit that the construction and selection of nucleic acid molecules that encode temperature-sensitive, non-cytopathic RNA-dependent RNA polymerases (*i.e.*, replicases) would not require one of ordinary skill in the art to make such predictions.

In order to obtain additional temperature-sensitive, non-cytopathic RNA-dependent RNA polymerases, one of ordinary skill in the art would most likely use a mutagenesis and screening approach. That is, rather than trying to predict which individual mutation or combination of mutations would result in the desired phenotype(s), one would simply subject a wild-type RNA-dependent RNA polymerase to mutagenesis (either random or site-directed), thereby creating a library of candidate molecules, and then screen for individual members of the library having the desired characteristics, *i.e.*, temperature-sensitivity and non-cytopathicity. In pursuing such a mutagenesis/screening approach, one of ordinary skill in the art would be guided by, among other things, the disclosure of known mutations in alphaviral replicases that confer non-cytopathic or temperature-sensitive phenotypes. *See, e.g.*, specification at page 21, line 23 through page 22, line 25. Such teachings would point to general regions within the replicase (*e.g.*, the nsP2 and nsP4 genes) where the skilled artisan could focus his or her mutagenesis efforts. Alternatively, a skilled artisan could perform mutagenesis (random or site-directed) on the entire polymerase-encoding nucleic acid molecule, or pursue an "alanine-scan" approach, for example, in which all of the charged residues are systematically changed to alanines.

Applicants note that, at the time the present invention was made, several methods were well known in the art for creating libraries of mutations in a given nucleic acid molecule. *See, e.g.*, Sambrook *et al.*, "Creating Many Mutations in a Defined Segment of DNA," in *Molecular Cloning, A Laboratory Manual*, Sambrook *et al.*, eds., Cold Spring

Harbor Laboratory Press, pp. 15.95-15.108 (1989) (copy attached herewith as Exhibit 1). Moreover, methods were also well known for testing alphaviral RNA polymerases for temperature sensitivity and/or non-cytopathicity. *See, e.g.*, Weiss *et al.*, *J. Virol. 33*:463-474 (1980); Dryga *et al.*, *Virology 228*:74-83 (1997); Burge and Pfefferkorn, *Virol. 30*:204-213 (1966); Burge and Pfefferkorn, *Virol. 30*:214-223 (1966) (copies of these documents were submitted as documents AR26, AS6, AR4 and AS4, respectively, with the Information Disclosure Statement filed on June 29, 1999). Therefore, the techniques needed to obtain additional temperature-sensitive, non-cytopathic RNA-dependent RNA polymerases were well within the abilities of persons of ordinary skill in the art at the time the present invention was made.

Experimentation, even complex experimentation, is not undue if the art typically engages in such experimentation. *See In re Certain Limited-Charge Cell Culture Microcarriers*, 221 U.S.P.Q. 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd. sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 U.S.P.Q. 428 (Fed. Cir. 1985); *see also In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). In *Wands*, for example, the Federal Circuit specifically stated that screening hybridomas to identify those that produce a desired antibody is not "undue experimentation" because "[p]ractitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody." *Wands* 858 F.2d at 740, 8 USPQ2d at 1406. Likewise, those skilled in the art would be prepared to screen for alphaviral replicases that are temperature-sensitive and non-cytopathic using mutagenesis and screening methods that are common in the art. Since persons of ordinary skill in the art routinely create libraries of mutations in particular nucleic acid molecules and screen for desired phenotypes of the

polypeptides encoded therefrom, it follows that the generation of nucleic acid molecules that encode temperature-sensitive, non-cytopathic RNA-dependent RNA polymerases would not be regarded as undue experimentation.

Applicants additionally note that claims 75, 103 and 125 have been amended to specify that, for the alphavirus replicases included within these claims, non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase. Thus, the claims themselves provide additional guidance as to where one of ordinary skill in the art would focus his or her mutagenesis efforts in order to obtain additional non-cytopathic, temperature-sensitive RNA-dependent RNA polymerases.

In view of the discussion above, Applicants respectfully request that the rejection of claims 75-79, 81-84, 86-101, 103, 105-107 and 109-136 under 35 U.S.C. § 112, first paragraph, for lack of enablement, be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the

number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,
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Version with markings to show changes made

75. (Twice amended) A DNA molecule which encodes an RNA molecule comprising:

(a) at least one *cis*-acting sequence element,

(b) a first open reading frame which encodes a non-cytopathic temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase, [RNA-dependent RNA polymerase of alphaviral origin,] and

(c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive alphaviral replicase [RNA-dependent RNA polymerase].

81. (Twice amended) The DNA molecule of claim 75, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Sindbis virus.

82. (Once amended) The DNA molecule of claim 75 which encodes an alphaviral replicase [RNA-dependent RNA polymerase] having replicase activity at 34°C which is at

least five fold lower than the replicase activity exhibited at 29°C.

86. (Once amended) A method of making a recombinant host cell comprising introducing the DNA molecule of claim 75 into a host cell *in vitro*.

93. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) introducing at least one DNA molecule of claim 75 into said host cells *in vitro*;

(b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

94. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) introducing at least one RNA molecule of claim 90 into said host cells *in vitro*;

(b) culturing said host cells under conditions suitable for expression of said protein or untranslated RNA molecule; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

97. (Once amended) A method for producing alphaviral particles, said method comprising:

(a) introducing into a host cell *in vitro* at least one DNA molecule of claim 75 having one or more open reading frames which encode alphaviral structural proteins;

(b) growing host cells under culture conditions suitable for the production of alphaviral particles which contain an RNA transcription product of said DNA molecule; and

(c) recovering said alphaviral particles.

98. (Once amended) A method for producing a protein encoded by RNA contained in an alphaviral particle produced by the method of claim 97 in a recombinant host cell comprising:

(a) infecting a host cell *in vitro* with the alphaviral particle;

(b) growing said host cell under culture conditions suitable for the production of said protein; and

(c) recovering said protein.

100. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one DNA molecule of claim 75 into said host cells *in vitro*; and

(c) changing the temperature of the host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

101. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one RNA molecule of claim 90 into said host cells *in vitro*; and

(c) changing the temperature of the host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

103. (Twice amended) A DNA vector system comprising one or more polynucleotides which encode RNA molecules, said RNA molecules comprising:

(a) at least one *cis*-acting sequence element,

(b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase [RNA-dependent RNA polymerase of alphaviral origin], and

(c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is recognized by said non-cytopathic, temperature-sensitive alphaviral replicase [RNA-dependent RNA polymerase].

105. (Once amended) The DNA vector system of claim 103 which encodes an alphaviral replicase [RNA-dependent RNA polymerase] having replicase activity at 34°C

which is at least five fold lower than the replicase activity exhibited at 29°C.

109. (Once amended) A method of making a recombinant host cell comprising introducing at least one polynucleotide of claim 103 into a host cell *in vitro*.

116. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one DNA molecule of claim 103 into said host cells *in vitro*;

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

117. (Once amended) A method for producing a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one RNA molecule of claim 113 into said host cells *in vitro*; and

(c) recovering said protein or untranslated RNA molecule;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

120. (Once amended) A method for producing an alphaviral particle comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing into said host cells *in vitro* at least one DNA molecule of claim 103 having one or more open reading frames which encode alphaviral structural proteins;

(c) producing an alphaviral particle; and

(e) recovering said alphaviral particle.

121. (Once amended) A method for producing a protein in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) infecting said host cells *in vitro* with an alphaviral particle produced by the method of claim 120; and

(c) recovering said protein;

wherein said protein is encoded by nucleic acid contained in said alphaviral particle.

123. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one DNA molecule of claim 103 into said host cells *in vitro*; and

(c) changing the temperature of the host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said DNA molecule.

124. (Once amended) A method for regulating the expression of a protein or an untranslated RNA molecule in a recombinant host cell comprising:

(a) growing host cells under suitable culture conditions;

(b) introducing at least one RNA molecule of claim 111 into said host cells *in vitro*; and

(c) changing the temperature of the host cell culture from:

(i) a permissive temperature to a restrictive temperature, or

(ii) a restrictive temperature to a permissive temperature;

wherein said protein or untranslated RNA molecule is encoded by said RNA molecule.

125. (Twice amended) A composition comprising one or more RNA molecules, said RNA molecules comprising:

(a) at least one *cis*-acting sequence element,

(b) a first open reading frame having a nucleotide sequence encoding a non-cytopathic, temperature-sensitive alphaviral replicase, wherein non-cytopathicity and temperature sensitivity are conferred by one or more mutations in the genes encoding the nonstructural proteins of said replicase [RNA-dependent RNA polymerase of alphaviral origin], and

(c) at least one second nucleotide sequence selected from the group consisting of:

(i) a second open reading frame encoding a protein, or portion thereof, wherein said second open reading frame is in a translatable format after one or more RNA-dependent RNA replication events;

(ii) a sequence complementary to all or part of the second open reading frame of (i); and

(iii) a sequence encoding an untranslated RNA molecule, or complement thereof;

wherein said second nucleotide sequence is operably linked to a promoter which is activated by said non-cytopathic, temperature-sensitive alphaviral replicase [RNA-dependent RNA polymerase].

126. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Semliki Forest Virus.

127. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from an Aura virus.

128. (Once amended) The DNA molecule of claim 75, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort [m]Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Triniti virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

129. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Sindbis virus.

130. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Semliki Forest Virus.

131. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from an Aura virus.

132. (Once amended) The DNA vector system of claim 103, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort [m]Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Triniti virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

133. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Sindbis virus.

134. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a Semliki Forest Virus.

135. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from an Aura virus.

136. (Once amended) The RNA molecule of claim 125, wherein said alphaviral replicase [the RNA-dependent RNA polymerase] is derived from a virus selected from the group consisting of Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalomyelitis virus, Fort [m]Morgan virus, Getah virus, Kyzylagach virus, Mayoaro virus, Middleburg virus, Mucambo virus, Ndumu virus, Pixuna virus, Tonate virus, Triniti virus, Una virus, Western equine encephalomyelitis virus, Whataroa virus, Venezuelan equine encephalomyelitis virus (VEE), and Ross River virus.

Claim 79 is sought to be cancelled.

Claims 137-145 are sought to be added.